EXHIBIT A

Attorney Docket: NEX87/C

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

APPLICANT: PAGRATIS *ET AL.*

SERIAL NO.: 09/791,301

FILED: FEBRUARY 23, 2001

TITLE: HIGH AFFINITY TGFβ NUCLEIC ACID LIGANDS AND INHIBITORS

EXAMINER: ZITOMER, S.W.

ART UNIT: 1634

CONFIRMATION NO: 9270

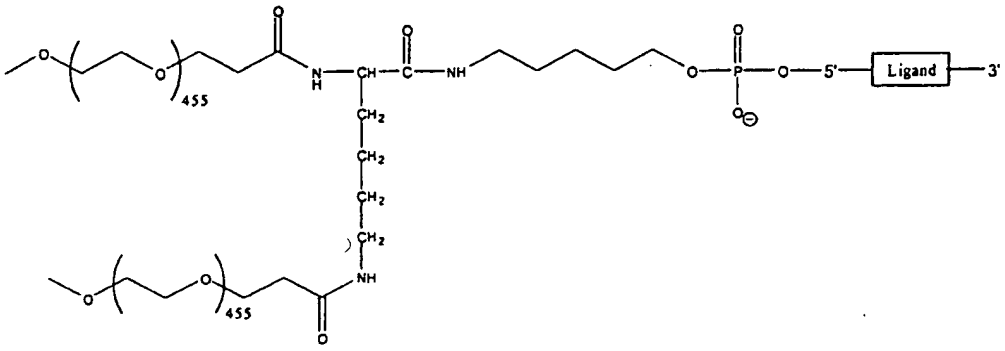
Assistant Secretary and Commissioner
of Patents and Trademarks
Washington, D.C. 20231

## AMENDMENT AND ELECTION WITH TRAVERSE

In the Claims:

Please enter the following amendment to Claim 8:

8. (Amended) The Complex of Claim 7 wherein said Complex is:




**LIGAND=**
5'mGmGmGrUrGrCrCrUrUrUrUrGrCrCrUmAmGmGrUrUmGrUmGmArUrU
rUmGrUmAmArCrCrUrUrCrUrGrCrCrCmA3'-3'rU (SEQ ID NO:210)

37 CFR 1.8
CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231 on November 30, 2005

Signature:
Name: Lisa S. Susser

Election with Traverse

In an Office Action mailed on April 10, 2002, the Examiner stated that the instant claims encompass 287 distinct inventions, each corresponding to a single nucleic acid ligand to TGF$\beta$1 or TGF$\beta$2. Applicants respectfully seek to traverse the restriction requirement by distinctly and specifically pointing out the errors therein in the following paragraphs.

Restriction is proper only when it is shown that a patent claims at least two independent or distinct inventions and that examination without restriction would place a serious burden on the Examiner. MPEP § 803. The Applicants respectfully submit that the Examiner has failed to demonstrate that a serious burden would be imposed if all 287 claimed sequences were examined in a single application. Applicants also respectfully submit that it is wholly unreasonable and inappropriate to apply current USPTO policy regarding examination of nucleic acid sequences to the instant nucleic acid ligands.

The Applicants would like to review for the Examiner the history of the promulgation of current USPTO policy regarding nucleic acid sequences. On March 12, 1996, the United States Patent and Trademark Office invited the public to comment on the problem created by the filing of patent applications claiming large numbers of nucleic acid sequences. *See 61 Fed. Reg. 9980.* Specifically, the USPTO noted that "scientific and technological advances have permitted researchers to identify large numbers of gene fragments rapidly." *Id.* The filing of patent applications claiming those gene fragments, termed Expressed Sequence Tags (ESTs), posed numerous problems for the USPTO due to the cost associated with the extensive database searches that must be performed during examination.

At a public hearing held on April 16, 1996, then-Commissioner Bruce Lehman noted that at least 70 patent applications comprising 200,000 claimed sequences were currently pending. Commissioner Lehman further pointed out that the number of patent applications was growing, and that "based on the number of organisms in [*sic* and] genes still to be discovered, such growth will continue for the near future."

In response to the comments received, the USPTO announced a new examination policy as follows:

> Nucleotide sequences encoding different proteins are structurally distinct chemical compounds and are unrelated to one another. These sequences are thus deemed to normally constitute independent and distinct inventions within the meaning of 35 U.S.C. 121. Absent evidence to the contrary, each such [coding] nucleotide sequence is presumed to represent an independent and distinct invention, subject to a restriction requirement pursuant to 35 U.S.C. 121 and 37 CFR 1.141.

*1992 O.G. 68*

From the above discussion, it is abundantly clear that the sole motivation for this policy came from the burden imposed on the USPTO through the filing of patent applications claiming hundreds and thousands of <u>different organismal coding</u> sequences, *i.e.*, patent applications that claim different genes and fragments from different genes (ESTs). Because such sequences encode different proteins, there is no unity of invention.

Nucleic acid ligands are non-coding sequences. Nucleic acid ligands are not isolated from any organism; they are, by definition, non-naturally occurring molecules. Each nucleic acid ligand is evolved by the SELEX process from a synthetic candidate mixture of randomized nucleic acid sequences. The SELEX process selects those nucleic acids in the candidate mixture that have the ability to bind to a particular target. Nucleic acid ligands are not selected for their ability to encode proteins. Any coding potential that a nucleic acid ligand may possess is fortuitous and plays no role whatsoever in the binding of the nucleic acid ligand to its cognate target. It, therefore, seems wholly unreasonable and inappropriate to apply a policy aimed at curbing

excessive numbers of different genes and different gene fragments to the subject nucleic acid ligands.

Furthermore, the USPTO policy is directed towards patent applications that claim multiple coding sequences, each coding for a different protein. Because the sequences code for different proteins, there is clearly no unity of invention. Nucleic acid ligands that bind a single target, in contrast, are unified in their function. This unity of function further distinguishes applications that claim nucleic acid ligands from applications that claim different coding sequences, and further illustrates the inappropriateness of requiring restriction in the former case. In the instant application, the nucleic acid ligands either bind to TGF$\beta$1 (SEQ ID NOS: 194-215) or TGF$\beta$2 (SEQ ID NOS: 21-121 and 128-193). Applicants submit that restriction to either nucleic acid ligands that bind TGF$\beta$1 or to nucleic acid ligands that bind to TGF$\beta$2 is appropriate, but that further restriction is not.

There are additional reasons for why it is inappropriate to apply the current restriction policy to nucleic acid ligands that bind the same target. For coding sequences, similarity must be determined both at the overt nucleotide level, and also at the amino acid level of the encoded protein. Each amino acid can be coded for by 1-6 different codons, so it is possible for identical proteins to be coded for by nucleotide sequences that bear as little as 70% identity to one another. For example, in the case of *In re Bell*, 991 F.2d 781 (Fed. Cir. 1993), the Court heard that the number of sequences that could potentially encode human IGF-1 would exceed $10^{36}$ due to this degeneracy of the genetic code. This creates additional search problems since gene patents frequently claim the disclosed sequences very broadly as explained below:

> For example, typical claims include the sequence and any sequence having a certain percentage identity or homology to the sequence or any sequence which hybridizes to the sequence, with or without the conditions of binding being recited. Others recite the sequence or any fragment of the sequence having a particular length of nucleotides. These claims are largely responsible for the lengthy search and evaluation times and the high resultant costs to the PTO.

*61 Fed. Reg. at 9980* (emphasis added).

Searches for nucleic acid ligands are far simpler than those for coding sequences. Since nucleic acid ligands do not code for proteins, and since only the disclosed sequences are claimed (not sequences with a certain percentage identity or homology to the claimed sequences, or sequences with polymorphic sites), the searches can be performed in a vastly shorter time and at greatly reduced cost in comparison with gene patent searches. The differences in the complexity of the searches once again illustrates the inappropriateness of adhering to the one sequence/patent guideline for nucleic acid ligands and clearly demonstrates that there is no serious burden on the Examiner.

Applicants respectfully point out that searching for nucleic acid sequences that exactly match those of the claimed nucleic acid ligands is, moreover, a futile endeavor. Since nucleic acid ligands are non-naturally occurring and are derived from randomized sequence, the likelihood of finding the same sequence in any genomic database is vanishingly small. Indeed, Applicants have not found in any sequenced prokaryotic or eukaryotic genome a single sequence that is identical to any nucleic acid expected to be found in the initial, unselected candidate mixtures from which the nucleic acid ligands claimed in the instant application were selected. Specifically, the Applicants searched all genomic sequences available at the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/BLAST/ ) with the BLAST algorithm using the following consensus sequence (corresponding to the vast majority sequences in Tables 5, 7, and 8 of the instant application) as the query:

5' GGGAGGACGAUGCGGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCAGACGACUCGCCCGA 3'

wherein N=A, G, C, or T, and wherein the underlined regions are the fixed sequence regions used to amplify the "winning" nucleic acids from each round of the SELEX process. In addition, the following concensus sequence (corresponding to all of the ligands in Tables 16 and 17) was also searched:

5' GGGAGGACGAUGCGGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCAGACGACUCGCCCGA 3'

In each case, while a small number of "hits" are found for each of the fixed regions alone, no hits are found for the complete consensus sequence. Applicants point out that this result indicates that no sequenced prokaryotic or eukaryotic genome contains a single sequence that is identical to any nucleic acid ligand that may be derived from the represented candidate mixtures.

Finally, Applicants have further searched all sequenced prokaryotic and eurkaryotic genomes for nucleic acids that have the following sequence, wherein "_" represents a gap of indeterminate length:

5' GGGAGGACGAUGCGG_CAGACGACUCGCCCGA 3'

Again, no hits are found, thereby indicating that no sequenced genome has a sequence that would correspond to those nucleic acids ligands in Tables 5, 7, and 8 that have fewer than 40 nucleotides between the fixed 5' and 3' regions.

Applicants respectfully suggest that the Patent and Trademark Office might usefully employ these search strategies in the future for nucleic acid ligands. Clearly, performing a single search cannot constitute a serious burden for the Examiner.

Because nucleic acid ligands do not occur in animal genomes, the only prior art sources that need be searched are those references and databases that list nucleic acid ligands to the same target as the subject application. This is a much smaller number of prior art sources in comparison to the number of sources that must be searched when a patent claims coding sequences and polymorphisms. Moreover, Applicants would also like to point out that even if there is a prior art description of nucleic acid ligands to the same targets as in the instant application, the likelihood that the prior art ligands will have the same sequence as the instant nucleic acid ligands is vanishingly small. In other words, if the SELEX process were to isolate a high affinity sequence to some target protein, it is almost impossible that a second *in vitro* selection, starting from an

independent candidate mixture, would isolate the same exact sequence. This will be demonstrated in the following paragraphs.

SELEX process candidate mixtures (also referred to as "libraries") are comprised of sequences with *N* nucleotides in the random region. Since there are 4 possible nucleotides at each position, there exist $4^N$ unique sequences in the *N*-random library. In the case where $N = 40$ (as in Tables 5, 7, and 8), this means that there are $4^{40} = 1.21 \times 10^{24}$ unique sequences. Typically, the SELEX process starts with an initial random library of $10^{14}$ individual sequences. We wish, then, to compute the probability that two independent libraries of $10^{14}$ sequences will contain at least one copy of the identical winning sequence given a total library complexity of $4^{40}$.

Given a sequence of interest, it is easier to compute the probability that <u>none</u> of the $10^{14}$ sequences in the starting library correspond to the sequence of interest. The probability that the first sequence is not the one of interest is simply:

$$p = (1 - \frac{1}{4^N}) \qquad (1)$$

The probability that <u>none</u> of the $10^{14}$ sequences is the sequence of interest is the joint probability that <u>each</u> sequence differs from the target one. This probability is the product of equation (1) over all the $10^{14}$ sequences in the starting pool:

$$\begin{aligned} p &= (1 - \frac{1}{4^N})(1 - \frac{1}{4^N})...(1 - \frac{1}{4^N}) \\ &= (1 - \frac{1}{4^N})^{10^{14}} \qquad (2) \end{aligned}$$

The probability that a pool will have <u>at least one copy</u> of the sequence of interest is then:

$$p = 1 - (1 - \frac{1}{4^N})^{10^{14}} \qquad (3)$$

Equation 3 is exact; however, it is untractable for computing probabilities here since the numbers are so large. A good numerical approximation to the probability in equation (2) may be derived when one realizes that the probability associated with a particular sequence is small, $4^{-N}$. Therefore, we can neglect all the cross products of equation (2), giving, to a first approximation, :

$$p \approx 1 - \frac{10^{14}}{4^N} \qquad (4)$$

The approximate probability of finding the sequence of interest at least once in the starting pool is then:

$$p \approx \frac{10^{14}}{4^N} \qquad (5)$$

In the instant application, wherein $N = 40$, the probability of finding the sequence of interest at least once in the starting pool according to equation (5) is $p = 8.3 \times 10^{-11}$. In other words, if one knows the sequence of a particular nucleic acid ligand of a target, one would need to perform more than 12 billion SELEX experiments against the same target in order to even find one copy of that same sequence in the initial candidate mixture. The probability of actually selecting that single copy of the sequence again as one of the "winning" nucleic acid ligands of the target after multiple rounds of the SELEX process is likely to be even more remote. This is because it is easy to lose a given nucleic acid, even one that binds tightly to a target, from the first round of the SELEX process when it is present only as a single copy. Hence, Applicants assert that even searching a database restricted to nucleic acid ligands to the same target (which in itself would be a far more restricted, and hence simpler, search than those for ESTs and polymorphisms) would also be a futile endeavor because the likelihood that the same nucleic acid ligand has been described before is almost infinitely remote.

Applicants also note that many of the sequences that are claimed in the instant application have the same sequences, but different nucleotide modifications. For example, Table 18 (comprising SEQ ID NO: 115 and SEQ ID NOS: 171-186) provides TGF$\beta$2 nucleic acid ligands that all have the following sequence:

GGAGGUUAUUACAGAGUCUGUAUAGCUGUACUCC [3'T]

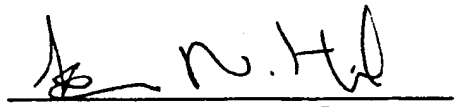
The individual sequences in Table 18 differ from one another only in the position of modified purines (substitution of 2'-OH with 2'-$OCH_3$). Similarly, Table 19 provides five TGF$\beta$2 nucleic acid ligands (SEQ ID NOS: 189-193) with identical sequence, but with variations in the position of 2'-$OCH_3$ modified purines. Finally, in Table 22 TGF$\beta$1 nucleic acid ligands corresponding to SEQ ID NOS: 194-211 are identical to one another in sequence, but have variations in the positions of 2'-$OCH_3$ modified purines. In each case where multiple nucleic acid ligands with the same sequence, but with different modification positions, are claimed, Applicants submit that it would be inappropriate to require restriction since only a single nucleotide sequence search would be performed. Again, a single search does not constitute a serious burden on the Examiner.

Applicants assert that it is inappropriate to require restriction as the Examiner has failed to even allege that a serious burden would be imposed by examination of the claims as filed. Moreover, Applicants have demonstrated that, unlike genes and gene fragments, multiple nucleic acid ligands can be examined without serious burden in a single application. Specifically, Applicants have shown that: a) it is futile to search for a particular nucleic acid ligand in any prior art database as the chance of finding the identical sequence in the prior art is infinitely remote; and b) a single search of a candidate mixture can be used to simultaneously search for all nucleic acid ligands derived from that candidate mixture. For these reasons, Applicants respectfully submit that the restriction requirement is in error and request reconsideration thereof.

Should the Examiner maintain the restriction requirement, Applicants pursuant to 37 C.F.R. § 1.143 hereby elect Claims 1-8 wherein said ligand is SEQ ID NO: 210 and request cancellation of the non-elected subject matter without prejudice either to the Applicants' right to file a divisional application at a later time, or to the Applicants' right to petition the Commissioner for reconsideration of the restriction requirement pursuant to 37 C.F.R. § 1.144.

This constitutes a request for any needed extension of time and an authorization to charge all fees therefore to deposit account No. 19-5117, if not otherwise specifically requested. The undersigned hereby authorizes the charge of any fees created by the filing of this document or any deficiency of fees submitted herewith to be charged to deposit account No. 19-5117.

Respectfully submitted,

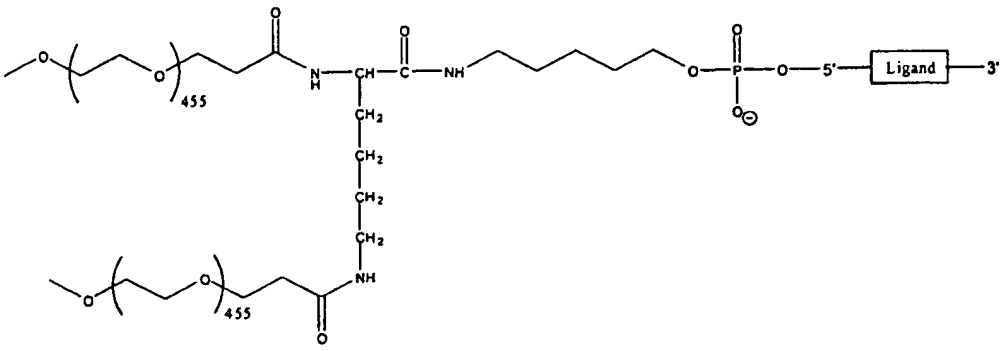


Steven N. Hird, #51,112
Swanson & Bratschun, L.L.C.
1745 Shea Center Drive, Suite 330
Highlands Ranch, Colorado 80129
(303) 268-0066

cc: John Harre
Diane Cruz
Vanessa Appleby

Marked up version showing changes to claims under 37 C.F.R. § 1.121(c)

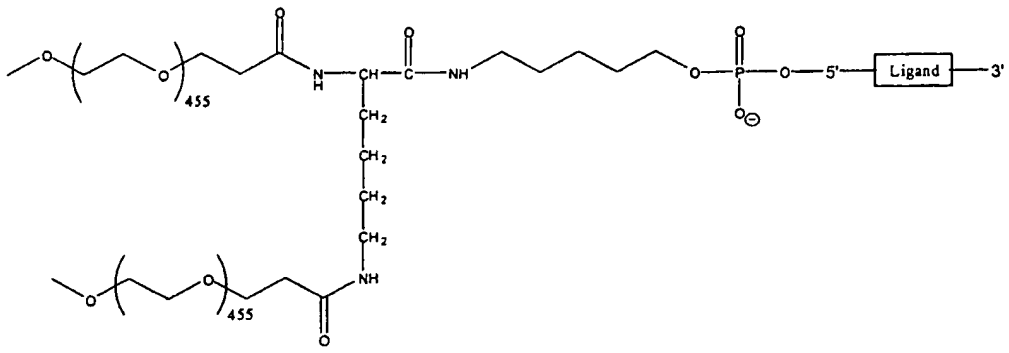
8. (Amended) The Complex of Claim 7 wherein said Complex is:




LIGAND=
~~rGrGrArGrGfUfUrAfUfUrAfCrArGrArGfUfCfUrGfUfUrArGfCfUrGfUrAfCfUfCfC-3'-3'-dT~~
5'mGmGmGrUrGrCrCrUrUrUrUrGrCrCrUmAmGmGrUrUmGrUmGmArUrU
rUmGrUmAmArCrCrUrUrCrUrGrCrCrCmA3'-3'rU (SEQ ID NO: 210)

Marked up version showing changes to claims under 37 C.F.R. § 1.121(c)

8. (Amended) The Complex of Claim 7 wherein said Complex is:




LIGAND=
~~rGrGrArGrGfUfUrAfUfUrAfCrArGrArGfUfCfUrGfUfUrArGfCfUrGfUrAfCfUfCfC-3'-3'-dT~~
5'mGmGmGrUrGrCrCrUrUrUrUrGrCrCrUmAmGmGrUrUmGrUmGmArUrU
rUmGrUmAmArCrCrUrUrCrUrGrCrCrCmA3'-3'rU (SEQ ID NO: 210)